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(54) Title: SPECIFIC PEPTIDE SUBSTRATE FOR HUMAN YAK1, YAK3A, AND YAK3B AND YEAST YAK1 PROTEIN KINASES

(57) Abstract: hYAK1, hYAK3a and 3b, and yeast YAK1 polypeptides and polynucleotides, methods for producing such polypeptides by recombinant techniques, and methods of using Ser-164 and these polypeptides and polynucleotides to determine the effect on phosphorylation are disclosed. Also disclosed are methods for utilizing hYAK1, hYAK3a and 3b, and yeast YAK1 polypeptides and polynucleotides in the design of protocols for the treatment of bone loss including osteoporosis; inflammatory diseases such as Adult Respiratory Disease Syndrome (ARDS), Rheumatoid arthritis, Osteoarthritis, Inflammatory Bowel Disease (IBD), psoriasis, dermatitis, asthma, allergies; infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; HIV-associated cachexia and other immunodeficiency disorders; septic shock; pain; injury; cancers; anorexia; bulimia; Parkinson's disease; cardiovascular disease including restenosis, atherosclerosis, acute heart failure, myocardial infarction; hypotension; hypertension; urinary retention; angina pectoris; ulcers; benign prostatic hypertrophy; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourett's syndrome, among others, and diagnostic assays for such conditions.

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SPECIFIC PEPTIDE SUBSTRATE FOR HUMAN YAK1, YAK3A, AND YAK3B AND YEAST YAK1 PROTEIN KINASES

Related Applications

This application claims priority of U.S. Provisional Application Serial No. 60/163,901, filed on November 5, 1999.

Field of the Invention

This invention relates to a newly identified peptide substrate for the serine/threonine protein kinase family members, human YAK1, human YAK3a and hYAK3b, and yeast YAK1. More particularly, the peptide of the present invention is derived from the carboxyl terminal end of bovine myelin basic protein (MBP). It is a 15 amino acid peptide with Ser at position 164 of the bovine MBP sequence as the major site of phosphorylation by hYAK1, hYAK3a and hYAK3b, and yeast YAK1, hereinafter referred to as Ser164. The sequence of Ser164 is:

Leu-Gly-Gly-Arg-Asp-Ser-Arg-Ser-Gly-Ser-Pro-Met-Ala-Arg-Arg (SEQ ID NO:9)

155 160 164 169

The invention also relates to a method of screening for inhibitors of human YAK1 and YAK3a and YAK3b, and yeast YAK1 protein serine/threonine kinases, and to inhibiting or activating the action of the above mentioned protein kinases and other YAK1 homologs by agents discovered using Ser164 or related peptide analogs as a method of screening for inhibitors and activators. In addition, this invention relates to using the Ser164 peptide to search for cellular substrates and/or cellular proteins that interact with the YAK family members of STPK's.

Background of the Invention

A number of polypeptide growth factors and hormones mediate their cellular effects through a signal transduction pathway. Transduction of signals from the cell surface receptors for these ligands to intracellular effectors frequently involves phosphorylation or dephosphorylation of specific protein substrates by regulatory protein serine/threonine kinases (PSTK) and phosphatases. Serine/threonine phosphorylation is a major mediator of signal transduction in multicellular organisms. Receptor-bound, membrane-bound and intracellular PSTKs regulate cell proliferation, cell differentiation and signaling processes in many cell types.

Aberrant protein serine/threonine kinase activity has been implicated or is suspected in a number of pathologies such as rheumatoid arthritis, psoriasis, septic shock, bone loss, many cancers and other proliferative diseases. Accordingly, serine/threonine kinases and the signal transduction

pathways which they are part of are potential targets for drug design.

Several PSTKs are involved in regulation of cell cycling. One example is the cyclin-dependent kinases or CDKs (Peter and Herskowitz, Cell 1994: 79, 181-184), which are activated by binding to regulatory proteins called cyclins and control passage of the cell through specific cell cycle checkpoints. For example, CDK2 complexed with cyclin E allows cells to progress through the G1 to S phase transition. The complexes of CDKs and cyclins are subject to inhibition by low molecular weight proteins such as p16 (Serrano et al, Nature 1993: 366, 704), which binds to and inhibits CDK4. Deletions or mutations in p16 have been implicated in a variety of tumors (Kamb et al, Science 1994: 264, 436-440). Therefore, the proliferative state of cells and diseases associated with this state are dependent on the activity of CDKs and their associated regulatory molecules. In diseases such as cancer where inhibition of proliferation is desired, compounds that inhibit CDKs may be useful therapeutic agents. Conversely, activators of CDKs may be useful where enhancement of proliferation is needed, such as in the treatment of immunodeficiency.

The yeast YAK1 is a PSTK with sequence homology to CDKs. It was originally identified in *S. cerevisiae* as a mediator of cell cycle arrest caused by inactivation of the cAMP-dependent protein kinase PKA (Garrett et al, Mol Cell Biol. 1991: 11, 4045-4052). YAK1 kinase activity is low in cycling yeast but increases dramatically when the cells are arrested prior to the S-G2 transition. Increased expression of YAK1 causes growth arrest in yeast cells deficient in PKA. Therefore, YAK1 can act as a cell cycle suppressor in yeast.

Frequently, in diseases such as osteoporosis and osteoarthritis, patients have established lesions of bone or cartilage, respectively. Treatment of such lesions requires an agent that will stimulate new bone or cartilage formation to replace that lost to the disease; therefore, there is a need for drugs that increase the number of osteoblasts or chondrocytes, the cells responsible for bone or cartilage formation, respectively. Similarly, replacement of heart or skeletal muscle depleted by diseases such as myocardial infarction or HIV-associated cachexia requires drugs that stimulate proliferation of cardiac myocytes or skeletal myoblasts.

This indicates that these protein serine/threonine kinases have an established, proven history as therapeutic targets. Clearly there is a need for identification and characterization of further members of the protein serine/threonine kinase family which can play a role in preventing, ameliorating or correcting dysfunctions or diseases, including, but not limited to, bone loss including osteoporosis; inflammatory diseases such as Adult Respiratory Disease Syndrome (ARDS), Rheumatoid arthritis, Osteoarthritis, Inflammatory Bowel Disease (IBD), psoriasis, dermatitis, asthma, allergies; infections such as bacterial, fungal, protozoan and viral infections, particularly infections

caused by HIV-1 or HIV-2; HIV-associated cachexia and other immunodeficiency disorders; septic shock; pain; injury; cancers; anorexia; bulimia; Parkinson's disease; cardiovascular disease including restenosis, atherosclerosis, acute heart failure, myocardial infarction; hypotension; hypertension; urinary retention; angina pectoris; ulcers; benign prostatic hypertrophy; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles dela Tourett's syndrome.

Previously, a novel human homolog of yeast YAK1, termed hYAK1, which is expressed in osteoblasts, chondrocytes, cardiac and skeletal muscle, and at lower levels, in placenta and pancreas was described (CREASY et al., EP Publication Number 98301124.8, published February 16, 1998, the disclosure of which is incorporated herein by reference in its entirety). The sequence of hYAK1 shares homology with predicted PSTK's from *C. elegans*, *S. pombe* and *S. cerevisiae* and has motifs associated with known protein kinases. Inhibitors of hYAK1 are expected to stimulate proliferation of cells in which it is expressed.

Another novel human homolog of yeast YAK1, termed hYAK3b, which is expressed in testis, skeletal muscle, and in hematopoietic cells that can undergo erythroid differentiation (XIE, and CREASY, U.S. Patent Number 5,965,420, granted October 12, 1999, and U.S. Application Serial Number 09/359,257, filed July 22, 1999, the disclosures of which are incorporated herein by reference in their entireties). The sequence of hYAK3b shares 79% amino acid identity with hYAK1 in its kinase domain, and shares homology with predicted PSTK's from *C. elegans*, *S. pombe* and *S. cerevisiae*.

hYAK1 and hYAK3b are members of a novel subfamily of protein kinases with unique structural and enzymatic features. This family had been termed Dyrk (for dual specificity YAK-related kinases) by Dr. Joost and his co-workers. In their publications, hYAK1 and hYAK3b were termed Dyrk2 and Dyrk3, respectively (Becker and Joost, Prog. Nucl. Acid Res., 1999, 62, 1-17).

Summary of the Invention

In one aspect, the invention relates to a peptide substrate for hYAK1, hYAK3a and hYAK3b and yeast YAK1. This peptide, which has the sequence LGGRDSRSGSPMARR(SEQ ID NO:9), is derived from the carboxyl terminal end of bovine MBP (residues 155-169), and in it the Ser residue at position 164 (underlined) was identified as the major phosphoacceptor residue for hYAK1. Hereinafter this peptide is referred to as Ser164. This same residue was identified as the major phosphoacceptor site also for hYAK3a and 3b and yeast YAK1 PSTKs. One aspect of the invention relates to methods for using the Ser164 peptide as a substrate to screen for inhibitors of the kinase activity of hYAK1, hYAK3a, hYAK3b, yeast YAK1, and other members of this family of

protein kinases. Such uses include the treatment of bone loss including osteoporosis; inflammatory diseases such as Adult Respiratory Disease Syndrome (ARDS), Rheumatoid arthritis, Osteoarthritis, Inflammatory Bowel Disease (IBD), psoriasis, dermatitis, asthma, allergies; infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; HIV-associated cachexia and other immunodeficiency disorders; septic shock; pain; injury; cancers; anorexia; bulimia; Parkinson's disease; cardiovascular disease including restenosis, atherosclerosis, acute heart failure, myocardial infarction; hypotension; hypertension; urinary retention; angina pectoris; ulcers; benign prostatic hypertrophy; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles dela Tourett's syndrome, among others. In still another aspect, the invention relates to methods to identify agonists and antagonists using the materials provided by the invention, and treating conditions associated with hYAK1, hYAK3a and 3b, and yeast YAK1 imbalance with the identified compounds. Yet another aspect of the invention relates to diagnostic assays for detecting diseases associated with inappropriate hYAK1, hYAK3a and 3b, and yeast YAK1 activity or levels. Still another aspect of the invention is the use of the S164 peptide sequence to identify cellular proteins that are phosphorylated by or interact with hYAK1, hYAK3a and 3b, and yeast YAK1.

Brief Description of the Drawings

Figure 1 shows the determination of hYAK1-catalyzed phosphorylation sites on bovine MBP.

Figure 2 shows the activity of hYAK1, hYAK3b, and yeast YAK1 against bovine MBP and the S164 peptide.

Figure 3 shows the results of steady state two substrate analysis to determine the kinetic constants of the kinase reaction of purified recombinant hYAK1 using the Ser164 as substrate.

Description of the Invention

Definitions

The following definitions are provided to facilitate understanding of certain terms used frequently herein.

"hYAK1" refers, among others, generally to a polypeptide having the amino acid sequence set forth in SEQ ID NO:4 or an allelic variant thereof.

"hYAK1 activity or hYAK1 polypeptide activity" or "biological activity of the hYAK1 or hYAK1 polypeptide" refers to the metabolic or physiologic function of said hYAK1 including similar activities or improved activities or these activities with decreased undesirable side-effects. Also included are antigenic and immunogenic activities of said hYAK1.

"hYAK1 gene" refers to a polynucleotide having the nucleotide sequence set forth in SEQ ID NO:3 or allelic variants thereof and/or their complements.

"hYAK3a" refers, among others, generally to a polypeptide having the amino acid sequence set forth in SEQ ID NO:6 or an allelic variant thereof.

"hYAK3a activity or hYAK3a polypeptide activity" or "biological activity of the hYAK3a or hYAK3a polypeptide" refers to the metabolic or physiologic function of said hYAK3a including similar activities or improved activities or these activities with decreased undesirable side-effects. Also included are antigenic and immunogenic activities of said hYAK3a.

"hYAK3a gene" refers to a polynucleotide having the nucleotide sequence set forth in SEQ ID NO:5 or allelic variants thereof and/or their complements.

"hYAK3b" refers, among others, generally to a polypeptide having the amino acid sequence set forth in SEQ ID NO:8 or an allelic variant thereof.

"hYAK3b activity or hYAK3b polypeptide activity" or "biological activity of the hYAK3b or hYAK3b polypeptide" refers to the metabolic or physiologic function of said hYAK3b including similar activities or improved activities or these activities with decreased undesirable side-effects. Also included are antigenic and immunogenic activities of said hYAK3b.

"hYAK3b gene" refers to a polynucleotide having the nucleotide sequence set forth in SEQ ID NO:7 or allelic variants thereof and/or their complements.

"Yeast YAK1" refers, among others, generally to a polypeptide having the amino acid sequence set forth in SEQ ID NO:2 or an allelic variant thereof.

"Yeast YAK1 activity or yYAK1 polypeptide activity" or "biological activity of the yYAK1 or yYAK1 polypeptide" refers to the metabolic or physiologic function of said yYAK1 including similar activities or improved activities or these activities with decreased undesirable side-effects. Also included are antigenic and immunogenic activities of said yYAK1.

"Yeast YAK1 gene" refers to a polynucleotide having the nucleotide sequence set forth in SEQ ID NO:1 or allelic variants thereof and/or their complements.

"Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of an Fab or other immunoglobulin expression library.

"Isolated" means altered "by the hand of man" from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

"Polynucleotide" generally refers to any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications has been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

"Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well-described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide

may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993 and Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter *et al.*, "Analysis for protein modifications and nonprotein cofactors", *Meth Enzymol* (1990) 182:626-646 and Rattan *et al.*, "Protein Synthesis: Posttranslational Modifications and Aging", *Ann NY Acad Sci* (1992) 663:48-62.

"Variant" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, or deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Identity" reflects a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, determined by comparing the sequences. In general, identity refers to an exact nucleotide to nucleotide or amino acid to amino acid correspondence of the two polynucleotide or two polypeptide sequences, respectively, over the length of the sequences being compared. For sequences where there is not an exact correspondence, a "% identity" may be determined. In general, the two sequences to be compared are aligned to give a maximum correlation between the sequences. This may include inserting "gaps" in either one or both sequences, to enhance the degree of alignment. A % identity may be determined over the whole length of each of the sequences being compared (so-called global alignment), that is particularly suitable for sequences of the same or very similar length, or over shorter, defined lengths (so-called local alignment), that is more suitable for sequences of unequal length.

"Similarity" is a further, more sophisticated measure of the relationship between two polypeptide sequences. In general, "similarity" means a comparison between the amino acids of two polypeptide chains, on a residue by residue basis, taking into account not only exact correspondences between a between pairs of residues, one from each of the sequences being compared (as for identity) but also, where there is not an exact correspondence, whether, on an evolutionary basis, one residue is a likely substitute for the other. This likelihood has an associated 'score' from which the "% similarity" of the two sequences can then be determined.

Methods for comparing the identity and similarity of two or more sequences are well known in the art. Thus for instance, programs available in the Wisconsin Sequence Analysis Package, version 9.1 (Devereux J., *et al, Nucleic Acids Res*, 12, 387-395, 1984, available from Genetics Computer Group, Madison, Wisconsin, USA), for example the programs BESTFIT and GAP, may be used to determine the % identity between two polynucleotides and the % identity and the % similarity between two polypeptide sequences. BESTFIT uses the "local homology" algorithm of Smith and Waterman (*J. Mol. Biol.*, 147:195-197, 1981, Advances in Applied Mathematics, 2, 482-489, 1981) and finds the best single region of similarity between two sequences. BESTFIT is more suited to comparing two polynucleotide or two polypeptide sequences that are dissimilar in length, the program assuming that the shorter sequence represents a portion of the longer. In comparison, GAP aligns two sequences, finding a "maximum similarity", according to the algorithm of Neddleman and Wunsch (*J. Mo.l Biol.*, 48, 443-453, 1970). GAP is more suited to comparing sequences that are approximately the same length and an alignment is expected over the entire length. Preferably, the parameters "Gap Weight" and "Length Weight" used in each program are 50 and 3, for polynucleotide sequences and 12 and 4 for polypeptide sequences, respectively.

Preferably, % identities and similarities are determined when the two sequences being compared are optimally aligned.

Other programs for determining identity and/or similarity between sequences are also known in the art, for instance the BLAST family of programs (Altschul S.F., *et al., J. Mol. Biol.*, 215, 403-410, 1990, Altschul S.F., *et al., Nucleic Acids Res.*, 25:389-3402, 1997, available from the National Center for Biotechnology Information (NCBI), Bethesda, Maryland, USA and accessible through the home page of the NCBI at www.ncbi.nlm.nih.gov) and FASTA (Pearson W R, *Methods in Enzymology*, 183: 63-99 (1990); Pearson W R and Lipman D.J., *Proc Nat Acad Sci USA*, 85: 2444-2448 (1988) (available as part of the Wisconsin Sequence Analysis Package).

Preferably, the BLOSUM62 amino acid substitution matrix (Henikoff S. and Henikoff J.G., *Proc. Nat. Acad Sci. USA*, 89: 10915-10919 (1992)) is used in polypeptide sequence comparisons including where nucleotide sequences are first translated into amino acid sequences before comparison.

Preferably, the program BESTFIT is used to determine the % identity of a query polynucleotide or a polypeptide sequence with respect to a polynucleotide or a polypeptide sequence of the present invention, the query and the reference sequence being optimally aligned and the parameters of the program set at the default value, as hereinbefore described.

Alternatively, for instance, for the purposes of interpreting the scope of a claim including mention of a "% identity" to a reference polynucleotide, a polynucleotide sequence having, for example, at least 95% identity to a reference polynucleotide sequence is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference sequence. Such point mutations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion. These point mutations may occur at the 5' or 3' terminal positions of the reference polynucleotide sequence or anywhere between these terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. In other words, to obtain a polynucleotide sequence having at least 95% identity to a reference polynucleotide sequence, up to 5% of the nucleotides of the in the reference sequence may be deleted, substituted or inserted, or any combination thereof, as hereinbefore described. The same applies *mutatis mutandis* for other % identities such as 96%, 97%, 98%, 99% and 100%.

For the purposes of interpreting the scope of a claim including mention of a "% identity" to a reference polypeptide, a polypeptide sequence having, for example, at least 95% identity to a reference polypeptide sequence is identical to the reference sequence except that the polypeptide

sequence may include up to five point mutations per each 100 amino acids of the reference sequence. Such point mutations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion. These point mutations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between these terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence. In other words, to obtain a sequence polypeptide sequence having at least 95% identity to a reference polypeptide sequence, up to 5% of the amino acids of the in the reference sequence may be deleted, substituted or inserted, or any combination thereof, as hereinbefore described. The same applies *mutatis mutandis* for other % identities such as 96%, 97%, 98%, 99%, and 100%.

A preferred meaning for "identity" for polynucleotides and polypeptides, as the case may be, are provided in (1) and (2) below.

(1) Polynucleotide embodiments further include an isolated polynucleotide comprising a polynucleotide sequence having at least a 95, 97 or 100% identity to the reference sequence of SEQ ID NO:1, (or SEQ ID NOS: 3, 5, or 7) wherein said polynucleotide sequence may be identical to the reference sequence of SEQ ID NO:1 or may include up to a certain integer number of nucleotide alterations as compared to the reference sequence, wherein said alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein said number of nucleotide alterations is determined by multiplying the total number of nucleotides in SEQ ID NO:1 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of nucleotides in SEQ ID NO:1, or:

$$n_n \leq x_n - (x_n \bullet y),$$

wherein n_n is the number of nucleotide alterations, x_n is the total number of nucleotides in SEQ ID NO:1, y is 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and $\bullet$ is the symbol for the multiplication operator, and wherein any non-integer product of x_n and y is rounded down to the nearest integer prior to subtracting it from x_n. Alterations of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2 may create nonsense, missense or frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by the polynucleotide following such alterations.

(2) Polypeptide embodiments further include an isolated polypeptide comprising a polypeptide having at least a 95, 97 or 100% identity to a polypeptide reference sequence of SEQ ID NO:2, (or SEQ ID NOS: 4, 6, or 8-18)wherein said polypeptide sequence may be identical to the reference sequence of SEQ ID NO:2 or may include up to a certain integer number of amino acid alterations as compared to the reference sequence, wherein said alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein said number of amino acid alterations is determined by multiplying the total number of amino acids in SEQ ID NO:2 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of amino acids in SEQ ID NO:2, or:

$$n_a \leq x_a - (x_a \bullet y),$$

wherein n_a is the number of amino acid alterations, x_a is the total number of amino acids in SEQ ID NO:2, y is 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and • is the symbol for the multiplication operator, and wherein any non-integer product of x_a and y is rounded down to the nearest integer prior to subtracting it from x_a.

Peptides of the Invention

In one aspect, the present invention relates to the Ser164 peptides. The Ser164 peptides include the peptide of the sequence in Figure 3; as well as peptide analogs which were made to study the structure activity relationship of Ser164 phosphorylation by these PSTK's. These analogs include but are not limited to peptides 1 to 8 below (modified residues are bolded):

Leu-Gly-Gly-Arg-Asp-Ser-Arg-Ser-Gly-Ser-Pro-Met-Ala-Arg-Arg (Ser164) (SEQ ID NO:9)

Leu-Gly-Gly-Arg-Asp-Ser-Arg-Ser-Gly-Ser-**Ala**-Met-Ala-Arg-Arg (Peptide 1) (SEQ ID NO:10)

Leu-Gly-Gly-Arg-Asp-Ser-Arg-Ser-Gly-**Thr**-Pro-Met-Ala-Arg-Arg (Peptide 2) (SEQ ID NO:11)

Leu-Gly-Gly-Arg-Asp-Ser-Arg-Ser-Gly-Ser-Pro-**Pro**-Ala-Arg-Arg (Peptide 3) (SEQ ID NO:12)

Leu-Gly-Gly-Arg-Asp-Ser-Arg-Ser-**Pro**-Ser-Pro-Met-Ala-Arg-Arg (Peptide 4) (SEQ ID NO:13)

Leu-Gly-Gly-Arg-Asp-Ser-Arg-Ser-**Pro**-Ser-**Ala**-Met-Ala-Arg-Arg (Peptide 5) (SEQ ID NO:14)

Leu-Gly-Gly-Arg-Asp-Ser-Arg-**Ala**-Gly-Ser-Pro-Met-Ala-Arg-Arg (Peptide 6) (SEQ ID NO:15)

Leu-Gly-Gly-Arg-Asp-**Ala**-Arg-Ser-Gly-Ser-Pro-Met-Ala-Arg-Arg (Peptide 7) (SEQ ID NO:16)

Leu-Gly-Gly-Arg-Asp-**Ala**-Arg-**Ala**-Gly-Ser-Pro-Met-Ala-Arg-Arg (Peptide 8) (SEQ ID NO:17)

Also included within the Ser164 peptides are truncated peptides analogs that do not have the first three residues (Leu-Gly-Gly).

Detection of Ser164 as the site on MBP phosphorylated by the YAKs PSTKs:

By screening several proteins as substrates, we determined that hYAK1, hYAK3a and 3b and yeast YAK1 preferred MBP as a substrate over casein, enolase, poly-Gly/Tyr, and several forms of histone. To identify the phosphorylation sites on bovine MBP catalyzed by each of the above mentioned protein kinases, bovine MBP was incubated for 30 minutes at 30 °C with 100 μM ATP, 10 mM MgCl2, and purified hYAK1, purified hYAK3b, or yeast YAK1 immunoprecipitated (using anti-HA Ab) from crude extracts of *Saccharomyces cerevisiae* transfected with HA-tagged yeast YAK1. Such an *in vitro* phosphorylated MBP was digested with endoproteinase Lys-C for 2 hours at 38 °C in 50 mM Tris pH 8.8. An aliquot of the unfractionated protein digest was analyzed by MALDI TOF for the presence of phosphorylated peptides. MALDI mass spectra (Annan, and Carr, (1996) *Anal. Chem.* **68,** 3413-3421) were recorded on a Micromass TofSpec SE single-stage, gridless reflectron time-of-flight mass spectrometer equipped with a time-lag-focusing source. Samples were prepared by mixing the digest with an equal volume of matrix solution which contained two internal mass standards at a concentration of 200 fmol/μl. A 0.5 μl aliquot of this sample/matrix solution was applied to the MALDI target. The matrix solution was 10 μg/ml α-Cyano-4-hydroxycinnamic acid in 50:50 ethanol:acetonitrile. Samples were then irradiated by 337 nm photons from a pulsed Laser Science nitrogen laser operating at 5 Hz. Typically, 20-50 laser shots were summed into a single mass spectrum. Potential phosphopeptide precursor ions were selected for post-source-decay (PSD) analysis using a Bradbury-Nielsen ion gate. Phosphopeptides were isolated by reversed phase HPLC using a 1mm i.d. C18 column. Peptides are eluted using acetonitrile:water:0.1% trifluoroacetic acid gradients at 50 μl/min. The column eluent was split post detector such that 5 μl/min was sent to a Micromass QTOF quadrupole time-of-flight mass spectrometer, and 45 μl/min went to a fraction collector taking one minute fractions. Phosphopeptides were sequenced by tandem MS (Verma, et al. (1997) *Science*, **278,** 455-560.) using the QTOF mass spectrometer equipped with a nanoelectrospray source (Wilm, and Mann, (1994) *Analytical Chem.* **66,** 4390-4399 1994).

MALDI-TOF analysis of 5% of the unfractionated endoproteinase Lys-C digests from phosphorylated and nonphosphorylated MBP revealed 2 apparent phosphate-containing peptides at

m/z 1571.78 (MH+) and 1682.80 (MH+) (Fig. 1(labelled A)), corresponding to peptides of molecular mass 1570.77 and 1681.79, respectively. The 1570 Da species is consistent with the mass of a peptide containing residues 91-104 from MBP plus one mole of phosphate (calculated mass, 1570.78 Da), whereas the 1681 Da peptide was consistent with the mass of the C-terminal Lys-C fragment from MBP incorporating residues 155-169 plus one mole of phosphate (calculated mass, 1681.78). Observation of the characteristic loss of H_3PO_4 (98 Da) from both peptides upon subsequent PSD analysis (Annan, and Carr, (1996) *Anal. Chem.* **68,** 3413-3421) confirmed that these peptides were, in fact, phosphorylated.

The Lys-C digests were fractionated by reverse phase HPLC using MS as an on-line readout of the fractions. Fractions containing the two putative phosphopeptides were analyzed by nanoES, and the appropriate precursor ions sequenced by collision-induced dissociation tandem mass spectrometry. The spectrum obtained for the 1570.9 peptide (Fig. 1(labelled B)) is consistent with two phosphorylated forms of the sequence NIVTPRTPPPSQGK (SEQ ID NO:18), which corresponds to residues 91-104 of bovine MBP. The major phosphorylation site was found to be Thr97. The 181.04 Da mass difference between the b_6 and b_7 ions indicates the presence of phosphothreonine at this position. A very small amount of phosphorylation was also found at Thr94. The evidence for phosphorylation at this second site was a series of $b_n\Delta$ ions starting at b_4 and ending at b_6. (The evidence for phosphorylation at either site would converge at the b7 ion.)

The tandem mass spectrum of the 1681 peptide (Fig. 1(labelled C)) is consistent with a monophosphorylated form of LGGRDSRSGSPMARR (SEQ ID NO:9), corresponding to the C-terminal residues 155-169 of bovine MBP. Although the tandem MS data clearly indicate that Ser160 is not phosphorylated, it was unable to distinguish between Ser162 or Ser164 as the sole site of phosphorylation in this peptide. Assignment of the phosphorylation site as Ser164 is based on the presence of an S/TP sequence at this site, which is analogous to the motif found at Thr94 and Thr97.

Phosphorylation of bovine MBP on Thr97 by the MAP kinases has been previously reported (Erikson, et al., (1990) *J. Biol. Chem.* **265,** 19728-19735;Clark-Lewis, et al., (1991) *J. Biol. Chem.* **266,** 11580-11584.). However, we are not aware of any kinase that phosphorylates bovine MBP on Ser164. Therefore, the phosphorylation pattern of yeast YAK1 on MBP appears to be unique.

Kinase Assays Using Ser164 as the phosphoacceptor

The source of Ser164 peptide: It was custom-ordered from California Peptide Research Inc. (Napa, CA), and its purity was determined by HPLC. The peptide contained 15 amino acids, and its

calculated molecular mass was 1601.82 daltons. Solid sample was dissolved at 5 mM in ice-cold kinase assay buffer (see later), aliquoted, and stored at –20 °C until use.

Figure 1 shows a determination of YAK1-induced phosphorylation sites on myelin basic protein. *A,* Partial MALDI mass spectrum of an unfractionated Lys-C digest of *in vitro* phosphorylated MBP. Peptides modified by a single mole of phosphate show an increase in mass of 80 Da. ***B,*** ES-CID tandem mass spectrum of the M^{2+} ion (m/z 786.4) from the 1570 Da phosphorylated peptide shown above. Ions marked $b_n\Delta$ have the structure b_n-H_3PO_4. ***C,*** ES-CID tandem mass spectrum of the M^{3+} ion (m/z 561.6) from the 1681 Da phosphorylated peptide shown above. Ions marked $b_n^{\Psi\Delta}$ have the structure b_n-H_3PO_4 –NH_3.

Figure 2 shows the activity of hYAK1, hYAK3, and yeast YAK1 against MBP and the Ser164 peptide. 5 ng purified hYAK1 and 100 ng purified hYAK3 were used per assay. Anti-HA mAb immune complex kinase assay was performed on 100 μg protein from crude extracts of yeast cells expressing either FL or ΔN yeast YAK1. Concentration of ATP was 100 μM, Ser164 was used at 0.5 mM, and MBP was at 10 μg/reaction (18.5 uM).

Figure 3 shows double reciprocal plots of enzyme velocity vs. ATP or Ser164 concentration. Steady state two substrate analysis of purified hYAK1 using S164 as phosphoacceptor. 1 ng hYAK1 was incubated for 30 minutes at 37 °C in hYAK1 reaction mix that contains various concentrations of ATP and Ser164. Each ATP concentration was used with 1, 0.75, 0.5, 0.33, 0.25, 0.15, and 0.05 mM Ser164. Each Ser164 concentration was used with 333, 166, 100, 66.7, 33.3, 16.7, and 6.6 μM ATP. Reciprocal values of enzyme velocity were calculated and plotted against reciprocal values of the concentration of each substrate. Data were analyzed by GraphFit, and calculated kinetic constants are shown.

The source of enzyme:

1) hYAK1: DET1/DET2-tagged full length hYAK1 was expressed in Drosophila sf9 cells and purified to >95% purity using Ni column chromatography. The purified protein migrated on SDS gels as a single band with an apparent molecular mass of 62 kDa. Samples were stored at –80 °C until use.

2) hYAK3b: Glutathione-S-Transferase (GST)/Factor Xa-tagged hYAK3b was expressed in baculovirus cells and purified to about 50% purity using Glutathione Sepharose 4B column chromatography, followed by Ni-NTA column chromatography. Samples were stored at –80 °C until use.

3) Yeast YAK1: Full length and an amino-terminally truncated (amino acids 148-807, termed ΔN) hemagglutinin (HA)-tagged yeast YAK1 was each expressed in a strain of *S. cerevisiae* lacking the endogenous *YAK1* gene and all three *PKA* genes. Cultures for experiments were grown in liquid Sc-His to an OD_{600} of at least 1.0, washed with Sc-His g/r, resuspended in Sc-His g/r to twice the original volume and grown for 16-24 hours at RT. Cells were washed once with H_2O and the pellets stored at –80 °C until use. To prepare lysates, cell pellets were thawed and resuspended at 1 ml/100 ml of original culture in lysis buffer (LB) containing 50 mM Tris pH 7.5, 150 mM NaCl, 10 μg/ml each aprotinin, leupeptin and TLCK, 0.1 mM PMSF, 50 mM NaF, 1 mM NaVanadate, 10 mM β-glycerophosphate. Following the addition of 0.5 ml sterile acid-washed glass beads, cells were disrupted via ten, 30 second intervals of vortexing. NP40 was added to a 2% final concentration followed by rocking at 4 °C for 30-50 minutes. Lysates were clarified by high-speed centrifugation, and the supernatants were stored at –80 °C until use. Each form of yeast YAK1 was immunoprecipitated from the detergent extracts using anti-HA mAb.

Immune Complex Protein Kinase Assay for Yeast YAK1: Yeast cellular extracts were immunoprecipitated by rocking overnight at 4°C with 4 μg of the anti-HA tag antibody and 100 μl of 20% suspension of protein A agarose (GIBCO-BRL) in LB that contained 1% NP-40. Samples were then washed twice with LB and once with basic kinase assay buffer (25 mM Hepes, pH 7.5; 1 mM DTT; 10 mM β-glycerol phosphate; 0.2 mM NaV). Washed immune complexes were suspended in 20 μl of basic kinase assay buffer that contained 0.1 mM ATP, 3 μCi of [γ-^{32}P]ATP, 10 mM $MgCl_2$, plus either bovine MBP or the Ser164 peptide. After incubation for 15 minutes at 30°C, the reactions were stopped by adding 20 μl of 0.15 M phosphoric acid. Phosphorylated substrates were isolated by spotting 20 μl of each sample on phosphocellulose (p81) filters. Filters were washed 3 times with 75 mM phosphoric acid followed by 3 times with H_2O, and counted for ^{32}P incorporation using a β-scintillation counter.

Kinase assay of purified hYAK1 and hYAK3: The assay was performed in 96 well Minisorp plates (Costar, Catalog No 3356). Reaction (in 30 μl volume) mixtures contained in final concentrations, 25 mM Hepes buffer, pH 7.5; 0.2 mM sodium vandate; 10 mM $MgCl_2$; 1 mM DTT; 10 mM β-glycerol phosphate; 0.1% BSA; 0.1 mM ATP, 2.5 μCi of [γ-^{32}P]ATP; purified hYAK1 (1-5 ng/assay), or purified hYAK3b (50-100 ng/assay); and either bovine MBP or the Ser164 peptide used at the concentrations indicated below and in the descriptions of the figures. Reactions were incubated for 20 minutes at 37°C, and were stopped by adding 10 μl of 0.3 M phosphoric acid.

Phosphorylated substrates were isolated by spotting 20 µl of the reaction on p81 filters, and processed as detailed earlier.

This same assay can be performed on a FlashPlate format in which the plate is coated with MBP or with the S164 peptide by incubation overnight at 4 °C in 100 µl of either substrate dissolved in Sodium Carbonate buffer, pH 8.8. When coating with MBP, a solution of 100 µg/ml MBP was used to coat wells with 100 µl (10 µg) MBP per well. When coating with Ser164, a solution of 0.4 mg/ml (0.25 mM) was used to coat wells with 100 µl (40 µg) Ser164 per well. An example of a FlashPlate assay protocol and typical results are given below:

FlashPlate (FP) Protocol

1. Coat Maxisorp plates with MBP or Ser164 as above.
2. Wash plates once with kinase assay buffer (KB): 25 mM Hepes, pH 7.5; 0.2 mM NaV; 10 mM β-glycerol phosphate; 1 mM Na pyrophosphate
3. Add enzyme (Ni-hYAK1, diluted in KB), DMSO or inhibitors (in KB) and keep on ice for 30 minutes
4. Add KB containing Mg/ATP to a [final] of 0.1 mM [γ-^{33}P]ATP and 10 mM $MgCl_2$
5. Incubate with shaking, 1-2 hrs, at room temperature
6. Aspirate and wash 6 times with 0.5 ml KB
7. Read ^{33}P incorporation in FP reader
8. Blank = No enzyme added
9. Reaction volume: 25, 50 or 100 µl
10. 0.5 or 1.0 µCi ^{33}P/0.1 mM ATP
11. MBP-FP better than basic FP (in house coating)
12. 37 °C incubation was not better (several time points)
13. Other incubation times at room temperature were not better

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Results: Each kinase phosphorylated the Ser164 peptide to much higher specific activity than MBP (Figure 2). Steady state kinetic constants of the hYAK1 reaction were generated by

varying both substrates simultaneously and fitting enzyme velocity as a function of each substrate concentration. Double reciprocal plots (1/V vs. 1/[Substrate]) with S164 peptide as the phosphate acceptor are shown in Figure 3. GraphFit analysis of the results generated the following steady state kinetic constants:

K_m[ATP]=42 ±7 μM .

K_m[S164]=160 ±14 μM.

V_{max} = 51 ±6 μmol/mg.

k_{cat}=160 ±19 min^{-1}.

Typical results of FlashPlate are shown below

FlashPlate Typical results

Signal to noise ratio:	>7 fold
Blanks:	30-80 cpm
[Ni-hYAK1]:	As low as 20 ng/reaction (5 nM) for 100 μl reactions
	As low as 8 ng/reaction (5 nM) for 25 μl reactions
^{33}P:	As low as 0.5 μCi
Kinase inhibitors:	Potency comparable to tube assay:
	SKF-108752 IC50: 0.19 μM (0.1 μg hYAK1)
	0.13 μM; 0.16 μM (0.3 μg hYAK1)
	K252a IC50: 0.552 μM; 0.427 μM (0.3 μg hYAK1)
Specific Activity:	At 20 ng enzyme, MBP gave 58 ±3 *(n = 6)*, and Ser164 gave 484 ±63 *(n = 6)* nmol/mg protein
DMSO:	No effect up to 3%
Variability:	<10% (between wells and from plate to plate)

Screening Assays

The hYAK1, hYAK3a and 3b, and yeast YAK1 polypeptides of the present invention may be employed in a screening process for compounds, which activate (agonists) or inhibit activation of (antagonists, or otherwise called inhibitors) the hYAK1, hYAK3a and 3b, and yeast YAK1

polypeptides of the present invention. Thus, polypeptides of the invention may also be used to assess or identify agonists or antagonists from, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These agonists or antagonists may be natural substrates, ligands, receptors, etc., as the case may be, of the polypeptides of the present invention; or may be structural or functional mimetics of the polypeptide of the present invention. See Coligan *et al.*, *Current Protocols in Immunology* 1(2): Chapter 5 (1991).

hYAK1, hYAK3a and 3b , and yeast YAK1 polypeptides are ubiquitous in the mammalian host and are responsible for many biological functions, including many pathologies. Accordingly, it is desirous to find compounds and drugs which stimulate hYAK1, hYAK3a and 3b, and yeast YAK1 polypeptide on the one hand and which can inhibit the function of hYAK1, hYAK3a and 3b, and yeast YAK1 polypeptide on the other hand. In general, agonists are employed for therapeutic and prophylactic purposes for such conditions as bone loss including osteoporosis; inflammatory diseases such as Adult Respiratory Disease Syndrome (ARDS), Rheumatoid arthritis, Osteoarthritis, Inflammatory Bowel Disease (IBD), psoriasis, dermatitis, asthma, allergies; infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; HIV-associated cachexia and other immunodeficiency disorders; septic shock; pain; injury; cancers; anorexia; bulimia; Parkinson's disease; cardiovascular disease including restenosis, atherosclerosis, acute heart failure, myocardial infarction; hypotension; hypertension; urinary retention; angina pectoris; ulcers; benign prostatic hypertrophy; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles dela Tourett's syndrome. Antagonists may be employed for a variety of therapeutic and prophylactic purposes for such conditions as bone loss including osteoporosis; inflammatory diseases such as Adult Respiratory Disease Syndrome (ARDS), Rheumatoid arthritis, Osteoarthritis, Inflammatory Bowel Disease (IBD), psoriasis, dermatitis, asthma, allergies; infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; HIV-associated cachexia and other immunodeficiency disorders; septic shock; pain; injury; cancers; anorexia; bulimia; Parkinson's disease; cardiovascular disease including restenosis, atherosclerosis, acute heart failure, myocardial infarction; hypotension; hypertension; urinary retention; angina pectoris; ulcers; benign prostatic hypertrophy; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles dela Tourett's syndrome.

In general, such screening procedures may involve using appropriate cells which express the hYAK1, hYAK3a and 3b, and yeast YAK1 polypeptides or respond to hYAK1, hYAK3a and 3b, and yeast YAK1 polypeptide of the present invention. Such cells include cells from mammals, yeast,

Drosophila or *E.coli*. Cells which express the hYAK1, hYAK3a and 3b, and yeast YAK1 polypeptides (or cell membrane containing the expressed polypeptides) or respond to hYAK1, hYAK3a and 3b, and yeast YAK1 polypeptides are then contacted with a test compound to observe binding, or stimulation or inhibition of a functional response. The ability of the cells, which were contacted with the candidate compounds, is compared with the same cells which were not contacted for hYAK1, hYAK3a and 3b, and yeast YAK1 activity.

The knowledge that the hYAK1, hYAK3a and 3b, and yeast YAK1 encodes a protein kinase suggests that recombinant forms can be used to establish a protein kinase activity. Typically this would involve the direct incubation of hYAK1, hYAK3a and 3b, and yeast YAK1 with a protein or peptide substrate in the presence of γ-^{32}P- ATP, followed by the measurement of radioactivity incorporated into the substrate by separation and counting. Separation methods include immunoprecipitation, conjugation of substrate to a bead allowing separation by centrifugation or determination of incorporation by scintillation proximity assay, SDS-PAGE followed by autoradiography or biosensor analysis. While the specific substrates are not yet known, candidates include hYAK1, hYAK3a and 3b, and yeast YAK1 themselves (autophosphorylation), myelin basic protein, casein, histone and HSP27. Other substances might be discovered by incubating hYAK1, hYAK3a and 3b, and yeast YAK1 with random peptides conjugated to solid supports or displayed on the surface of phage or by incubation of hYAK1, hYAK3a and 3b, and yeast YAK1 with mammalian cell lysates and γ-^{32}P- ATP, followed by separation of the labelled target proteins, and sequencing. The protein kinase activity of hYAK1, hYAK3a and 3b, and yeast YAK1 may require incubation with a specific upstream effector. This may be achieved by preincubating hYAK1, hYAK3a and 3b, and yeast YAK1 with lysates from a variety of stimulated eukaryotic cells and ATP. These assays permit the discovery and modification of compounds which inhibit hYAK1, hYAK3a and 3b, and yeast YAK1 kinase activity in vitro and would be expected to have effects on proliferation of osteoblasts, chondrocytes, cardiac myocytes or skeletal myoblasts. Any inhibitors so identified would be expected to have up-regulatory effects on proliferation and be useful as a therapeutic for the treatment and prevention of diseases such as osteoporosis, osteoarthritis, cardiomyopathy and cachexia.

This invention contemplates the treatment and/or amelioration of such diseases by administering a hYAK1, hYAK3a and 3b, or yeast YAK1 inhibiting amount of a compound. Without wishing to be bound by any particular theory of the functioning of the hYAK1, hYAK3a and 3b, and yeast YAK1 polypeptides of this invention, it is believed that among the useful inhibitors of hYAK1, hYAK3a and 3b, and yeast YAK1 function are those compounds which inhibit the kinase activity of the hYAK1, hYAK3a and 3b, and yeast YAK1. Other sites of inhibition are, of course, possible

owing to its position in a signal transduction cascade. Therefore, inhibiting the interaction of hYAK1, hYAK3a and 3b, and yeast YAK1 with one or more of its upstream or downstream modulators/substrates is also contemplated by this invention. Inhibitors of protein-protein interactions between hYAK1, hYAK3a and 3b, and yeast YAK1 and other factors could lead to the development of pharmaceutical agents for the modulation of hYAK1, hYAK3a and 3b, and yeast YAK1 activity.

The assays may simply test binding of a candidate compound wherein adherence to the cells bearing the hYAK1, hYAK3a and 3b, and yeast YAK1 polypeptide is detected by means of a label directly or indirectly associated with the candidate compound or in an assay involving competition with a labeled competitor. Further, these assays may test whether the candidate compound results in a signal generated by activation of the hYAK1, hYAK3a and 3b, and yeast YAK1 polypeptide, using detection systems appropriate to the cells bearing the hYAK1, hYAK3a and 3b, and yeast YAK1 polypeptide. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist by the presence of the candidate compound is observed. Standard methods for conducting such screening assays are well understood in the art.

Examples of potential hYAK1, hYAK3a and 3b, and yeast YAK1 polypeptide antagonists include antibodies or, in some cases, oligonucleotides or proteins which are closely related to the ligands, substrates, receptors, etc., as the case may be, of the hYAK1, hYAK3a and 3b, and yeast YAK1 polypeptide, e.g., a fragment of the ligands, substrates, receptors, or small molecules which bind to the polypeptide of the present invention but do not elicit a response, so that the activity of the polypeptide is prevented.

Prophylactic and Therapeutic Methods

This invention provides methods of treating an abnormal condition related to both an excess of and insufficient amounts of hYAK1, hYAK3a and 3b, and yeast YAK1 polypeptide activity.

If the activity of hYAK1, hYAK3a and 3b, and yeast YAK1 polypeptides is in excess, several approaches are available. One approach comprises administering to a subject an inhibitor compound (antagonist) as hereinabove described along with a pharmaceutically acceptable carrier in an amount effective to inhibit activation by blocking binding of ligands to the hYAK1, hYAK3a and 3b, and yeast YAK1 polypeptides, or by inhibiting a second signal, and thereby alleviating the abnormal condition.

In another approach, soluble forms of hYAK1, hYAK3a and 3b, and yeast YAK1 polypeptides still capable of binding the ligand in competition with endogenous hYAK1, hYAK3a

and 3b, and yeast YAK1 polypeptides may be administered. Typical embodiments of such competitors comprise fragments of the hYAK1, hYAK3a and 3b, and yeast YAK1 polypeptides.

In still another approach, expression of the gene encoding endogenous hYAK1, hYAK3a and 3b, and yeast YAK1 polypeptide can be inhibited using expression blocking techniques. Known such techniques involve the use of antisense sequences, either internally generated or separately administered. See, for example, O'Connor, *J Neurochem* (1991) 56:560 in Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Alternatively, oligonucleotides which form triple helices with the gene can be supplied. See, for example, Lee *et al., Nucleic Acids Res* (1979) 6:3073; Cooney *et al., Science* (1988) 241:456; Dervan *et al., Science* (1991) 251:1360. These oligomers can be administered *per se* or the relevant oligomers can be expressed *in vivo*.

For treating abnormal conditions related to an under-expression of hYAK1, hYAK3a and 3b, and yeast YAK1 and its activity, several approaches are also available. One approach comprises administering to a subject a therapeutically effective amount of a compound which activates hYAK1, hYAK3a and 3b, and yeast YAK1 polypeptide, i.e., an agonist as described above, in combination with a pharmaceutically acceptable carrier, to thereby alleviate the abnormal condition. Alternatively, gene therapy may be employed to effect the endogenous production of hYAK1, hYAK3a and 3b, and yeast YAK1 by the relevant cells in the subject. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector, as discussed above. The retroviral expression construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells *in vivo* and expression of the polypeptide *in vivo*. For overview of gene therapy, see Chapter 20, *Gene Therapy and other Molecular Genetic-based Therapeutic Approaches*, (and references cited therein) in Human Molecular Genetics, T Strachan and A P Read, BIOS Scientific Publishers Ltd (1996).

Formulation and Administration

Peptides, such as the soluble form of hYAK1, hYAK3a and 3b, and yeast YAK1 polypeptides, and agonists and antagonist peptides or small molecules, may be formulated in combination with a suitable pharmaceutical carrier. Such formulations comprise a therapeutically effective amount of the polypeptide or compound, and a pharmaceutically acceptable carrier or excipient. Such carriers include but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The formulation should suit the mode of administration,

and is well within the skill of the art. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

Preferred forms of systemic administration of the pharmaceutical compositions include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if properly formulated in enteric or encapsulated formulations, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels and the like.

The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages, however, are in the range of 0.1-100 μg/kg of subject. Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

Polypeptides used in treatment can also be generated endogenously in the subject, in treatment modalities often referred to as "gene therapy" as described above. Thus, for example, cells from a subject may be engineered with a polynucleotide, such as a DNA or RNA, to encode a polypeptide *ex vivo*, and for example, by the use of a retroviral plasmid vector. The cells are then introduced into the subject.

Examples

The examples below are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. The examples illustrate, but do not limit the invention.

Example 1

A partial clone (ATG-355, HGS EST # 454640) was initially identified through random searches of the Human Genome Sciences database. This partial clone (~ 1kb) showed significant

homology to YAK1 from S cerevisiae. To get the full length cDNA: A total of 1M plaques were screened from a Human Osteoblast cDNA library (Stratagene, LaJolla CA) using the insert of the above partial clone as a probe. Library screening procedure is described by (Elgin, et al. Strategies 4: 8-9, 1991). The probes were α -32P labeled, using Random Primed Labeling Kit (Boheringer Manheim, Germany, Cat. # 1585584) and purified by running over Sephadex G-50 columns (Pharmacia Biotech. Cat. # 17-0855-02) The hybridization and washing conditions were according to (J. Sambrook, E.F. Fritch and T. Maniatis (1989) A Laboratory Manual Second. Ed. Vol. 1 pp. 2.69-2.81 Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York). Five clones were isolated by plaque purification and a fragments containing the inserts were excised and sequenced. The longest insert was a 2.7 kb fragment containing the 3' untranslated region and part of the coding sequence of hYAK1. A second probe prepared by EcoRI-BamHI digestion of this insert followed by labelling with 32P was used to screen a commercially available human heart cDNA library (Stratagene #936207). An additional five clones were plaque purified, excised into phagemids and sequenced. Fasta analysis show this peptide to have high homology to a putative serine/threonine kinase of unknown function from C. elegans (F49E11.1).

To confirm that the cDNA was full length, Human Leukocyte "Marathon Ready" cDNA (Clontech, Palo Alto, CA) was used as a template to amplify a fragment corresponding to the 5' region of hYAK1 using a 5' anchor primer-1 (Clontech) and a reverse gene specific primer. This fragment was T/A cloned into pCR2.1 (Invitrogen), and multiple isolates were sequenced. An in-frame stop codon was identified upstream of the predicted initiation codon confirming that the full-length cDNA had been obtained.

Northern analysis was carried out to determine the distribution of hYAK1 mRNA in human tissues. A fragment containing the 3' untranslated region of hYAK1 was isolated from SEQ ID NO:1 using standard techniques. The isolated fragment was radiolabelled with α-^{32}P-dATP using a randomly primed labelling kit. Membranes containing mRNA from multiple human tissues (Clontech #7760-1) were hybridized with the probe and washed under high stringency conditions as directed. Hybridized mRNA was visualized by exposing the membranes for 4 days to X-ray film. Three major transcripts of 2.6, 7 and 10 kb were present and were expressed most prominently in heart and skeletal muscle, but were present to a lesser degree in pancreas, placenta and brain. All three transcripts appeared absent from kidney.

SEQUENCE INFORMATION

SEQ ID NO:1

ATGAACTCAT CCAATAATAA CGACTCGTCC AGCTCCAATA GCAACATGAA TAACTCCTTG
AGCCCGACCC TTGTGACCCA CAGTGATGCT AGTATGGGCT CGGGTAGAGC AAGCCCAGAC
AATAGCCATA TGGGGAGAGG TATATGGAAT CCATCGTACG TAAATCAAGG CTCTCAAAGG
TCTCCACAGC AGCAGCATCA GAATCATCAC CAGCAGCAGC AGCAGCAGCA ACAACAACAA
CAACAGAATT CTCAATTTTG CTTTGTTAAC CCTTGGAATG AGGAAAAAGT AACTAATTCT
CAACAAAACC TGGTGTATCC CCCTCAATAC GATGACTTAA ACAGTAACGA AAGTCTAGAT
GCGTACAGAC GACGTAAATC TAGTCTCGTT GTACCTCCAG CCAGGGCACC TGCCCCAAAT
CCTTTCCAGT ACGATAGTTA TCCCGCTTAC ACCAGCTCTA ATACGAGTTT GGCAGGAAAT
AGCAGTGGCC AGTATCCTTC TGGCTATCAA CAACAACAAC AGCAAGTATA CCAGCAGGGC
GCTATCCATC CTTCCCAATT TGGATCCAGA TTTGTTCCCT CCCTTTATGA TCGTCAAGAT
TTCCAAAGAA GGCAGAGTCT GGCTGCAACT AATTATTCGT CCAATTTTTC TTCCCTAAAT
TCAAATACTA ATCAAGGAAC AAACTCCATA CCAGTCATGT CACCTTACAG GAGGCTTAGC
GCATATCCTC CTAGTACAAG TCCCCCACTA CAACCCCCTT TCAAGCAGTT ACGAAGAGAT
GAAGTACAAG GTCAGAAATT GTCCATTCCT CAGATGCAAC TTTGCAACTC TAAAAACGAT
CTTCAACCTG TCTTAAATGC TACTCCAAAG TTTAGACGTG CGTCGCTCAA TTCCAAGACG
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SEQ ID NO:11

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SEQ ID NO:12

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SEQ ID NO:13

Leu Gly Gly Arg Asp Ser Arg Ser **Pro** Ser Pro Met Ala Arg Arg (Peptide 4)

SEQ ID NO:14

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SEQ ID NO:15

Leu Gly Gly Arg Asp Ser Arg **Ala** Gly Ser Pro Met Ala Arg Arg (peptide 6)

SEQ ID NO:16

Leu Gly Gly Arg Asp **Ala** Arg Ser Gly Ser Pro Met Ala Arg Arg (Peptide 7)

SEQ ID NO:17

Leu Gly Gly Arg Asp **Ala** Arg **Ala** Gly Ser Pro Met Ala Arg Arg (peptide 8)

SEQ ID NO:18

NIVTPRTPPPSQGK

What is claimed is:

1. A method for screening for an agent that modulates a YAK polypeptide kinase activity comprising:

a. contracting a candidate agent with a YAK polypeptide, wherein the step of contacting is carried out under conditions and for a time sufficient to allow the candidate agent and the polypeptide to interact; and

b. subsequently measuring the ability of the candidate agent to modulate the ability of the polypeptide to phosphorylate the substrate.

2. The method of claim 1 wherein the YAK polypeptide has an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

3. The method of claim 2 wherein the substrate comprises the SER-164 polypeptide.

Figure 1

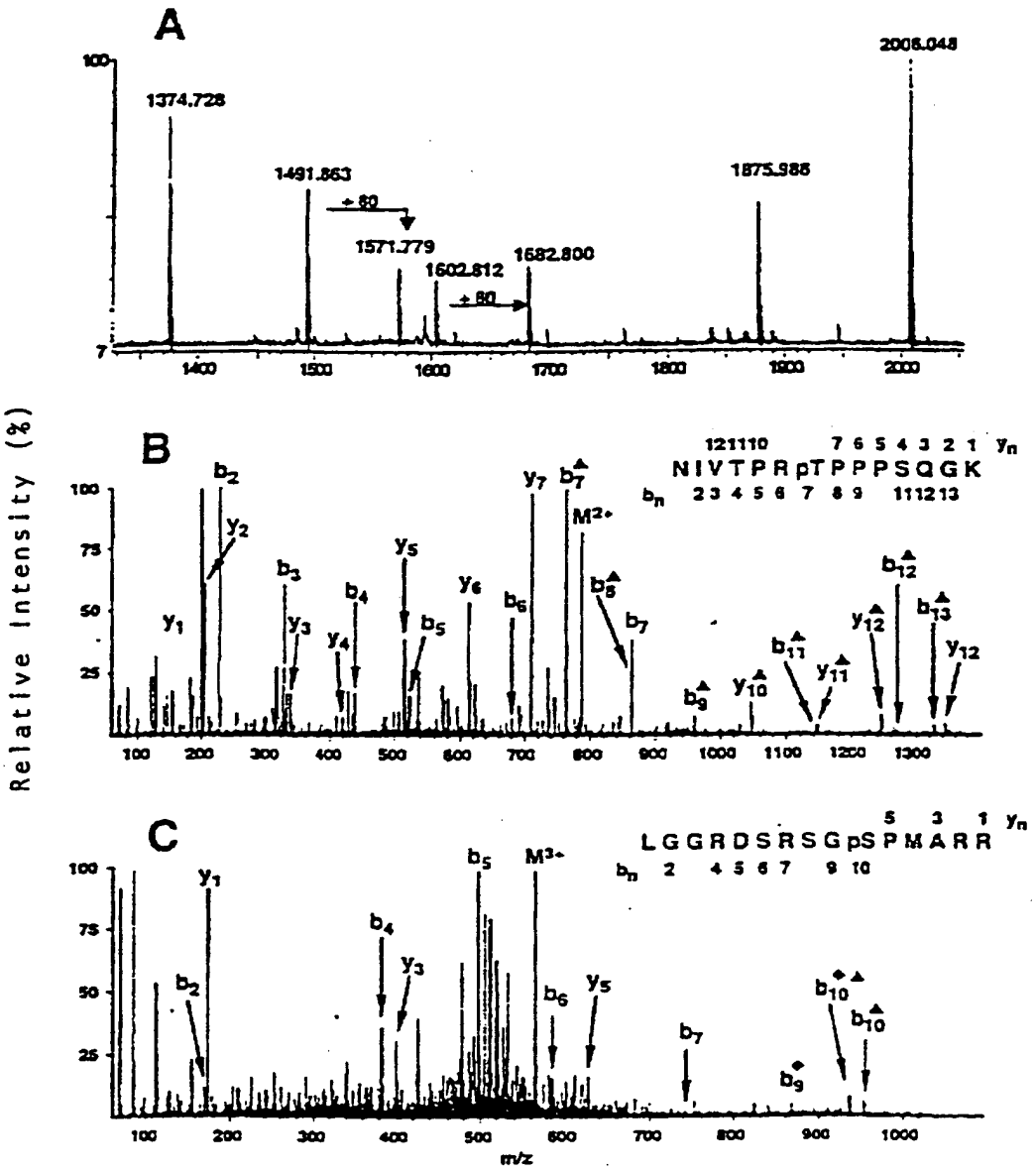


Figure 2

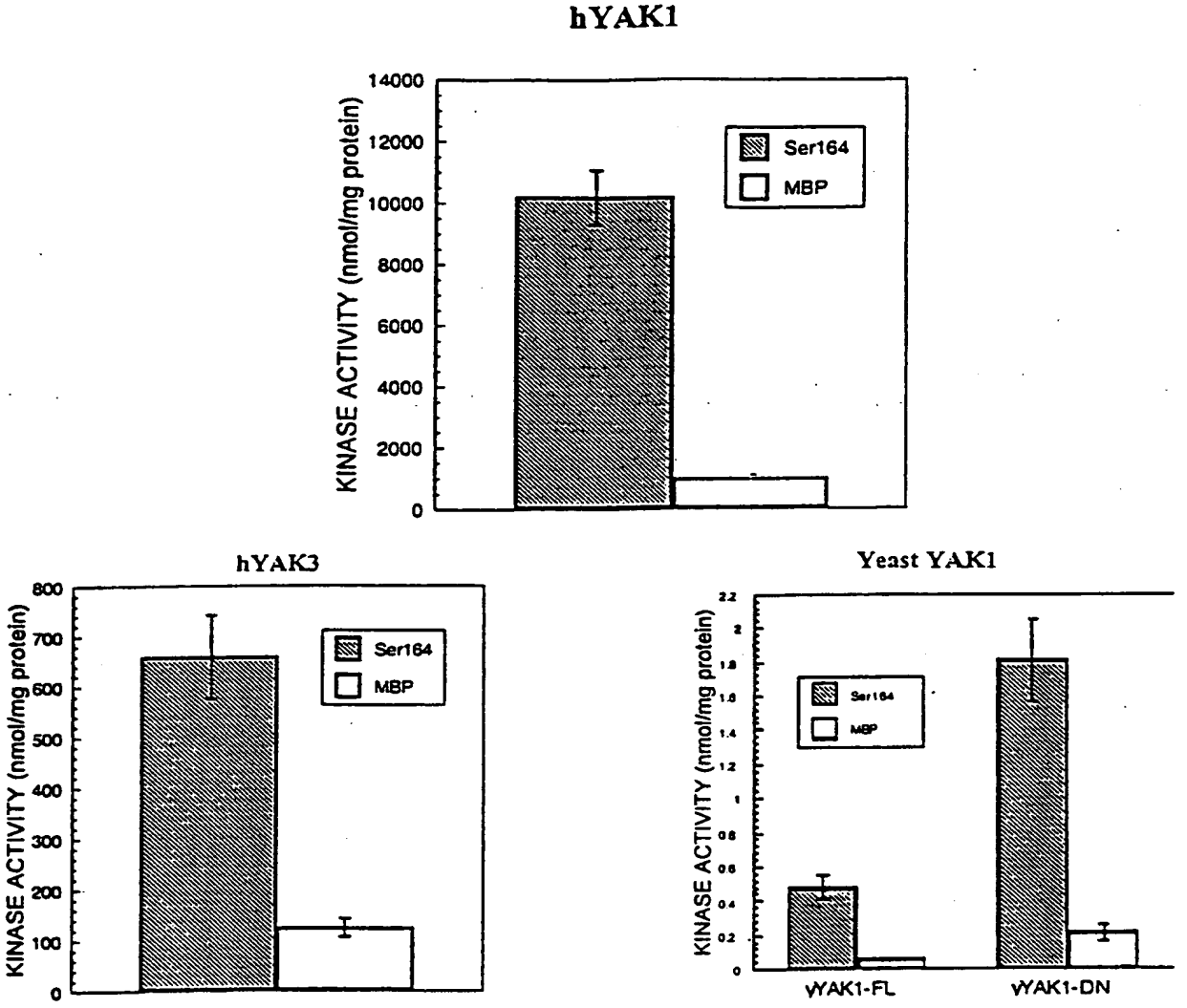
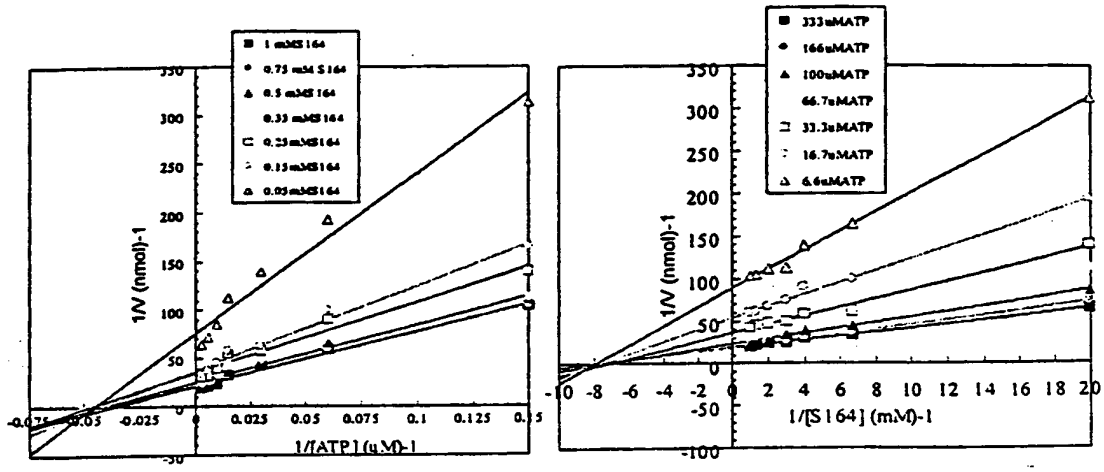


Figure 3

Steady State Two Substrate Kinetic Analysis



Kinetic Constants (GraphFit) :

K_m[ATP] = 42 ±7 uM K_m[S164] = 160 ±14 uM

V_{max} = 51 ±6 umol/mg k_{cat} = 160 ±19 min^{-1}

SEQUENCE LISTING

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SMITHKLINE BEEHCAM PLC

<120> SPECIFIC PEPTIDE SUBSTRATE FOR HUMAN YAK1, YAK3A, AND YAK3B AND YEAST YAK1 PROTEIN KINASES

<130> GH-70650WO

<140> TO BE ASSIGNED
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<150> 60/163,901
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tattttaagt ggagaaagtt accagatatt attagaaact acaggtatcc taaaagcata   1980
cagaactccc aagaacttat cgaccaagaa atgcagaata gggagtgttt gatccacttt   2040
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cacccttca taacaaagca ggagtttaca ggtgagtggt ttcctccagg atcgtcttta   2160
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<210> 2
<211> 807
<212> PRT
<213> HOMO SAPIENS

<400> 2

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            20                  25                  30
Gly Ser Gly Arg Ala Ser Pro Asp Asn Ser His Met Gly Arg Gly Ile
        35                  40                  45
Trp Asn Pro Ser Tyr Val Asn Gln Gly Ser Gln Arg Ser Pro Gln Gln
    50                  55                  60
Gln His Gln Asn His His Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln
65                  70                  75                  80
Gln Gln Asn Ser Gln Phe Cys Phe Val Asn Pro Trp Asn Glu Glu Lys
                85                  90                  95
Val Thr Asn Ser Gln Gln Asn Leu Val Tyr Pro Pro Gln Tyr Asp Asp
            100                 105                 110
Leu Asn Ser Asn Glu Ser Leu Asp Ala Tyr Arg Arg Arg Lys Ser Ser
        115                 120                 125
Leu Val Val Pro Pro Ala Arg Ala Pro Ala Pro Asn Pro Phe Gln Tyr
    130                 135                 140
Asp Ser Tyr Pro Ala Tyr Thr Ser Ser Asn Thr Ser Leu Ala Gly Asn
145                 150                 155                 160
Ser Ser Gly Gln Tyr Pro Ser Gly Tyr Gln Gln Gln Gln Gln Gln Val
                165                 170                 175
Tyr Gln Gln Gly Ala Ile His Pro Ser Gln Phe Gly Ser Arg Phe Val
            180                 185                 190
Pro Ser Leu Tyr Asp Arg Gln Asp Phe Gln Arg Arg Gln Ser Leu Ala
        195                 200                 205
Ala Thr Asn Tyr Ser Ser Asn Phe Ser Ser Leu Asn Ser Asn Thr Asn
    210                 215                 220
Gln Gly Thr Asn Ser Ile Pro Val Met Ser Pro Tyr Arg Arg Leu Ser
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Ala Tyr Pro Pro Ser Thr Ser Pro Pro Leu Gln Pro Pro Phe Lys Gln
                245                 250                 255
Leu Arg Arg Asp Glu Val Gln Gly Gln Lys Leu Ser Ile Pro Gln Met
            260                 265                 270
Gln Leu Cys Asn Ser Lys Asn Asp Leu Gln Pro Val Leu Asn Ala Thr
        275                 280                 285
Pro Lys Phe Arg Arg Ala Ser Leu Asn Ser Lys Thr Ile Ser Pro Leu
    290                 295                 300
Val Ser Val Thr Lys Ser Leu Ile Thr Thr Tyr Ser Leu Cys Ser Pro
305                 310                 315                 320
Glu Phe Thr Tyr Gln Thr Ser Lys Asn Pro Lys Arg Val Leu Thr Lys
                325                 330                 335
Pro Ser Glu Gly Lys Cys Asn Asn Gly Phe Asp Asn Ile Asn Ser Asp
            340                 345                 350
Tyr Ile Leu Tyr Val Asn Asp Val Leu Gly Val Glu Gln Asn Arg Lys
        355                 360                 365
Tyr Leu Val Leu Asp Ile Leu Gly Gln Gly Thr Phe Gly Gln Val Val
    370                 375                 380
Lys Cys Gln Asn Leu Leu Thr Lys Glu Ile Leu Ala Val Lys Val Val
385                 390                 395                 400
Lys Ser Arg Thr Glu Tyr Leu Thr Gln Ser Ile Thr Glu Ala Lys Ile
                405                 410                 415
Leu Glu Leu Leu Asn Gln Lys Ile Asp Pro Thr Asn Lys His His Phe
            420                 425                 430
Leu Arg Met Tyr Asp Ser Phe Val His Lys Asn His Leu Cys Leu Val
        435                 440                 445
Phe Glu Leu Leu Ser Asn Asn Leu Tyr Glu Leu Leu Lys Gln Asn Lys
    450                 455                 460
Phe His Gly Leu Ser Ile Gln Leu Ile Arg Thr Phe Thr Thr Gln Ile
465                 470                 475                 480
Leu Asp Ser Leu Cys Val Leu Lys Glu Ser Lys Leu Ile His Cys Asp
                485                 490                 495
Leu Lys Pro Glu Asn Ile Leu Leu Cys Ala Pro Asp Lys Pro Glu Leu
            500                 505                 510
Lys Ile Ile Asp Phe Gly Ser Ser Cys Glu Glu Ala Arg Thr Val Tyr
        515                 520                 525
Thr Tyr Ile Gln Ser Arg Phe Tyr Arg Ala Pro Glu Ile Ile Leu Gly
    530                 535                 540
Ile Pro Tyr Ser Thr Ser Ile Asp Met Trp Ser Leu Gly Cys Ile Val
545                 550                 555                 560
Ala Glu Leu Phe Leu Gly Ile Pro Ile Phe Pro Gly Ala Ser Glu Tyr
                565                 570                 575
Asn Gln Leu Thr Arg Ile Ile Asp Thr Leu Gly Tyr Pro Pro Ser Trp
            580                 585                 590
Met Ile Asp Met Gly Lys Asn Ser Gly Lys Phe Met Lys Lys Leu Ala
        595                 600                 605
Pro Glu Glu Ser Ser Ser Ser Thr Gln Lys His Arg Met Lys Thr Ile
    610                 615                 620
Glu Glu Phe Cys Arg Glu Tyr Asn Ile Val Glu Lys Pro Ser Lys Gln
625                 630                 635                 640
Tyr Phe Lys Trp Arg Lys Leu Pro Asp Ile Ile Arg Asn Tyr Arg Tyr
                645                 650                 655
Pro Lys Ser Ile Gln Asn Ser Gln Glu Leu Ile Asp Gln Glu Met Gln
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660 665 670
Asn Arg Glu Cys Leu Ile His Phe Leu Gly Gly Val Leu Asn Leu Asn
675 680 685
Pro Leu Glu Arg Trp Thr Pro Gln Gln Ala Met Leu His Pro Phe Ile
690 695 700
Thr Lys Gln Glu Phe Thr Gly Glu Trp Phe Pro Pro Gly Ser Ser Leu
705 710 715 720
Pro Gly Pro Ser Glu Lys His Asp Asp Ala Lys Gly Gln Gln Ser Glu
725 730 735
Tyr Gly Ser Ala Asn Asp Ser Ser Asn Asn Ala Gly His Asn Tyr Val
740 745 750
Tyr Asn Pro Ser Ser Ala Thr Gly Gly Ala Asp Ser Val Asp Ile Gly
755 760 765
Ala Ile Ser Lys Arg Lys Glu Asn Thr Ser Gly Asp Ile Ser Asn Asn
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Phe Ala Val Thr His Ser Val Gln Glu Gly Pro Thr Ser Ala Phe Asn
785 790 795 800
Lys Leu His Ile Val Glu Glu
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<210> 3
<211> 2085
<212> DNA
<213> HOMO SAPIENS

<400> 3
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gtgggcaaaa cgggcttgcc agtggtgcca gagcggcagc tggacagcat tcatagacgg 240
caggggagct ccacctctct aaagtccatg gaaggcatgg ggaaggtgaa agccaccccc 300
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<210> 4
<211> 528
<212> PRT
<213> HOMO SAPIENS

<400> 4

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            20                  25                  30
Thr Gln Pro Asn Gly Leu Thr Thr Val Gly Lys Thr Gly Leu Pro Val
        35                  40                  45
Val Pro Glu Arg Gln Leu Asp Ser Ile His Arg Arg Gln Gly Ser Ser
    50                  55                  60
Thr Ser Leu Lys Ser Met Glu Gly Met Gly Lys Val Lys Ala Thr Pro
65                  70                  75                  80
Met Thr Pro Glu Gln Ala Met Lys Gln Tyr Met Gln Lys Leu Thr Ala
                85                  90                  95
Phe Glu His His Glu Ile Phe Ser Tyr Pro Glu Ile Tyr Phe Leu Gly
            100                 105                 110
Leu Asn Ala Lys Lys Arg Gln Gly Met Thr Gly Gly Pro Asn Asn Gly
        115                 120                 125
Gly Tyr Asp Asp Asp Gln Gly Ser Tyr Val Gln Val Pro His Asp His
    130                 135                 140
Val Ala Tyr Arg Tyr Glu Val Leu Lys Val Ile Gly Lys Gly Ser Phe
145                 150                 155                 160
Gly Gln Val Val Lys Ala Tyr Asp His Lys Val His Gln His Val Ala
                165                 170                 175
Leu Lys Met Val Arg Asn Glu Lys Arg Phe His Arg Gln Ala Ala Glu
            180                 185                 190
Glu Ile Arg Ile Leu Glu His Leu Arg Lys Gln Asp Lys Asp Asn Thr
        195                 200                 205
Met Asn Val Ile His Met Leu Glu Asn Phe Thr Phe Arg Asn His Ile
    210                 215                 220
Cys Met Thr Phe Glu Leu Leu Ser Met Asn Leu Tyr Lys Leu Ile Lys
225                 230                 235                 240
Lys Asn Lys Phe Gln Gly Phe Ser Leu Pro Leu Val Arg Lys Phe Ala
                245                 250                 255
His Ser Ile Leu Gln Cys Leu Asp Ala Leu His Lys Asn Arg Ile Ile
            260                 265                 270
His Cys Asp Leu Lys Pro Glu Asn Ile Leu Leu Lys Gln Gln Gly Arg
        275                 280                 285
Ser Gly Ile Lys Val Ile Asp Phe Gly Ser Ser Cys Tyr Glu His Gln
    290                 295                 300
Arg Val Tyr Thr Tyr Ile Gln Ser Arg Phe Tyr Arg Ala Pro Glu Val
305                 310                 315                 320
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Ile Leu Gly Ala Arg Tyr Gly Met Pro Ile Asp Met Trp Ser Leu Gly
                325                 330                 335
Cys Ile Leu Ala Glu Leu Leu Thr Gly Tyr Pro Leu Leu Pro Gly Glu
            340                 345                 350
Asp Glu Gly Asp Gln Leu Ala Cys Met Ile Glu Leu Leu Gly Met Pro
        355                 360                 365
Ser Gln Lys Leu Leu Asp Ala Ser Lys Arg Ala Lys Asn Phe Val Ser
    370                 375                 380
Ser Lys Gly Tyr Pro Arg Tyr Cys Thr Val Thr Thr Leu Ser Asp Gly
385                 390                 395                 400
Ser Val Val Leu Asn Gly Gly Arg Ser Arg Arg Gly Lys Leu Arg Gly
                405                 410                 415
Pro Pro Glu Ser Arg Glu Trp Gly Asn Ala Leu Lys Gly Cys Asp Asp
            420                 425                 430
Pro Leu Phe Leu Asp Phe Leu Lys Gln Cys Leu Glu Trp Asp Pro Ala
        435                 440                 445
Val Arg Met Thr Pro Gly Gln Ala Leu Arg His Pro Trp Leu Arg Arg
    450                 455                 460
Arg Leu Pro Lys Pro Pro Thr Gly Glu Lys Thr Ser Val Lys Arg Ile
465                 470                 475                 480
Thr Glu Ser Thr Gly Ala Ile Thr Ser Ile Ser Lys Leu Pro Pro Pro
                485                 490                 495
Ser Ser Ser Ala Ser Lys Leu Arg Thr Asn Leu Ala Gln Met Thr Asp
            500                 505                 510
Ala Asn Gly Asn Ile Gln Gln Arg Thr Val Leu Pro Lys Leu Val Ser
        515                 520                 525
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<210> 5
<211> 2061
<212> DNA
<213> HOMO SAPIENS

<400> 5

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cccgcgcggg gtcccgagct aggagatggg aggcacagct cgtgggcctg ggcggaagga     180
tgcggggccg cctggggccg ggctcccgcc ccagcagcgg aggttggggg atggtgtcta     240
tgacaccttc atgatgatag atgaaaccaa atgtcccccc tgttcaaatg tactctgcaa     300
tccttctgaa ccacctccac ccagaagact aaatatgacc actgagcagt ttacaggaga     360
tcatactcag cacttttttgg atggaggtga gatgaaggta gaacagctgt ttcaagaatt     420
tggcaacaga aaatccaata ctattcagtc agatggcatc agtgactctg aaaaatgctc     480
tcctactgtt tctcagggta aaagttcaga ttgcttgaat acagtaaaat ccaacagttc     540
atccaaggca cccaaagtgg tgcctctgac tccagaacaa gccctgaagc aatataaaca     600
ccacctcact gcctatgaga aactggaaat aattaattat ccagaaattt actttgtagg     660
tccaaatgcc aagaaaagac atggagttat tggtggtccc aataatggag ggtatgatga     720
tgcagatggg gcctatattc atgtacctcg agaccatcta gcttatcgat atgaggtgct     780
gaaaattatt ggcaagggga gttttgggca ggtggccagg gtctatgatc acaaacttcg     840
acagtacgtg gccctaaaaa tggtgcgcaa tgagaagcgc tttcatcgtc aagcagctga     900
ggagatccgg attttggagc atcttaagaa acaggataaa actggtagta tgaacgttat     960
ccacatgctg gaaagtttca cattccggaa ccatgtttgc atggcctttg aattgctgag    1020
catagacctt tatgagctga ttaaaaaaaa taagtttcag ggttttagcg tccagttggt    1080
acgcaagttt gcccagtcca tcttgcaatc tttggatgcc ctccacaaaa ataagattat    1140
tcactgcgat ctgaagccag aaaacattct cctgaaacac cacgggcgca gttcaaccaa    1200
ggtcattgac tttgggtcca gctgtttcga gtaccagaag ctctacacat atatccagtc    1260
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tcggttctac agagctccag aaatcatctt aggaagccgc tacagcacac caattgacat 1320
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ggatgaagga gaccagttgg cctgcatgat ggagcttcta gggatgccac caccaaaact 1440
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ctctgtgact acccaggcag atgggagggt tgtgcttgtg gggggtcgct cacgtagggg 1560
taaaaagcgg ggtccccccag gcagcaaaga ctgggggaca gcactgaaag ggtgtgatga 1620
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cccagctcaa gcattaagac acccttggat tagcaagtct gtccccagac ctctcaccac 1740
catagacaag gtgtcaggga aacgggtagt taatcctgca agtgctttcc agggattggg 1800
ttctaagctg cctccagttg ttggaatagc caataagctt aaagctaact taatgtcaga 1860
aaccaatggt agtatacccc tatgcagtgt attgccaaaa ctgattagct agtggacaga 1920
gatatgccca gagatgcata tgtgtatatt tttatgatct tacaaacctg caaatggaaa 1980
aaatgcaagc ccattggtgg atgtttttgt tagagtagac ttttttttaaa caagacaaaa 2040
catttttata tgattataaa a 2061

<210> 6
<211> 588
<212> PRT
<213> HOMO SAPIENS

<400> 6

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            20                  25                  30
Asp Thr Phe Met Met Ile Asp Glu Thr Lys Cys Pro Pro Cys Ser Asn
        35                  40                  45
Val Leu Cys Asn Pro Ser Glu Pro Pro Pro Pro Arg Arg Leu Asn Met
    50                  55                  60
Thr Thr Glu Gln Phe Thr Gly Asp His Thr Gln His Phe Leu Asp Gly
65                  70                  75                  80
Gly Glu Met Lys Val Glu Gln Leu Phe Gln Glu Phe Gly Asn Arg Lys
                85                  90                  95
Ser Asn Thr Ile Gln Ser Asp Gly Ile Ser Asp Ser Glu Lys Cys Ser
            100                 105                 110
Pro Thr Val Ser Gln Gly Lys Ser Ser Asp Cys Leu Asn Thr Val Lys
        115                 120                 125
Ser Asn Ser Ser Ser Lys Ala Pro Lys Val Val Pro Leu Thr Pro Glu
    130                 135                 140
Gln Ala Leu Lys Gln Tyr Lys His His Leu Thr Ala Tyr Glu Lys Leu
145                 150                 155                 160
Glu Ile Ile Asn Tyr Pro Glu Ile Tyr Phe Val Gly Pro Asn Ala Lys
                165                 170                 175
Lys Arg His Gly Val Ile Gly Gly Pro Asn Asn Gly Gly Tyr Asp Asp
            180                 185                 190
Ala Asp Gly Ala Tyr Ile His Val Pro Arg Asp His Leu Ala Tyr Arg
        195                 200                 205
Tyr Glu Val Leu Lys Ile Ile Gly Lys Gly Ser Phe Gly Gln Val Ala
    210                 215                 220
Arg Val Tyr Asp His Lys Leu Arg Gln Tyr Val Ala Leu Lys Met Val
225                 230                 235                 240
Arg Asn Glu Lys Arg Phe His Arg Gln Ala Ala Glu Glu Ile Arg Ile
                245                 250                 255
Leu Glu His Leu Lys Lys Gln Asp Lys Thr Gly Ser Met Asn Val Ile
```

```
            260                 265                 270
His Met Leu Glu Ser Phe Thr Phe Arg Asn His Val Cys Met Ala Phe
        275                 280                 285
Glu Leu Leu Ser Ile Asp Leu Tyr Glu Leu Ile Lys Lys Asn Lys Phe
    290                 295                 300
Gln Gly Phe Ser Val Gln Leu Val Arg Lys Phe Ala Gln Ser Ile Leu
305                 310                 315                 320
Gln Ser Leu Asp Ala Leu His Lys Asn Lys Ile Ile His Cys Asp Leu
                325                 330                 335
Lys Pro Glu Asn Ile Leu Leu Lys His His Gly Arg Ser Ser Thr Lys
            340                 345                 350
Val Ile Asp Phe Gly Ser Ser Cys Phe Glu Tyr Gln Lys Leu Tyr Thr
        355                 360                 365
Tyr Ile Gln Ser Arg Phe Tyr Arg Ala Pro Glu Ile Ile Leu Gly Ser
    370                 375                 380
Arg Tyr Ser Thr Pro Ile Asp Ile Trp Ser Phe Gly Cys Ile Leu Ala
385                 390                 395                 400
Glu Leu Leu Thr Gly Gln Pro Leu Phe Pro Gly Glu Asp Glu Gly Asp
                405                 410                 415
Gln Leu Ala Cys Met Met Glu Leu Leu Gly Met Pro Pro Pro Lys Leu
            420                 425                 430
Leu Glu Gln Ser Lys Arg Ala Lys Tyr Phe Ile Asn Ser Lys Gly Ile
        435                 440                 445
Pro Arg Tyr Cys Ser Val Thr Thr Gln Ala Asp Gly Arg Val Val Leu
    450                 455                 460
Val Gly Gly Arg Ser Arg Arg Gly Lys Lys Arg Gly Pro Pro Gly Ser
465                 470                 475                 480
Lys Asp Trp Gly Thr Ala Leu Lys Gly Cys Asp Asp Tyr Leu Phe Ile
                485                 490                 495
Glu Phe Leu Lys Arg Cys Leu His Trp Asp Pro Ser Ala Arg Leu Thr
            500                 505                 510
Pro Ala Gln Ala Leu Arg His Pro Trp Ile Ser Lys Ser Val Pro Arg
        515                 520                 525
Pro Leu Thr Thr Ile Asp Lys Val Ser Gly Lys Arg Val Val Asn Pro
    530                 535                 540
Ala Ser Ala Phe Gln Gly Leu Gly Ser Lys Leu Pro Pro Val Val Gly
545                 550                 555                 560
Ile Ala Asn Lys Leu Lys Ala Asn Leu Met Ser Glu Thr Asn Gly Ser
                565                 570                 575
Ile Pro Leu Cys Ser Val Leu Pro Lys Leu Ile Ser
            580                 585
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<210> 7
<211> 2327
<212> DNA
<213> HOMO SAPIENS

<400> 7

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ctccccccat ccctgtctca ccgggcgcgg gggacggggc tagagcggag ttagagcaag     180
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acgaaaatct aggacttttt gtggggcttt ttattaaagg gggggagccc gggagcaata     300
ccttggaaag aagccctgtt gcttagagcg gataaccaac ggctgaactc ttggggtttg     360
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ctgtgagggg tgcggtctag cttcgaatgt acagtggtgg agccacagtg ttaaagaaca     420
gagaagtgat ccttaatcat ttagaatttt gcctccacca tccaccagaa aatgaagtgg     480
aaagagaagt tgggggatgg tgtctatgac accttcatga tgatagatga aaccaaatgt     540
ccccccctgtt caaatgtact ctgcaatcct tctgaaccac ctccacccag aagactaaat     600
atgaccactg agcagtttac aggagatcat actcagcact ttttggatgg aggtgagatg     660
aaggtagaac agctgtttca agaatttggc aacagaaaat ccaatactat tcagtcagat     720
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Val Glu Gln Leu Phe Gln Glu Phe Gly Asn Arg Lys Ser Asn Thr Ile
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Tyr Ile His Val Pro Arg Asp His Leu Ala Tyr Arg Tyr Glu Val Leu
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Val Gln Leu Val Arg Lys Phe Ala Gln Ser Ile Leu Gln Ser Leu Asp
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Arg Cys Leu His Trp Asp Pro Ser Ala Arg Leu Thr Pro Ala Gln Ala
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/30542

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C12Q 1/48; C12N 9/12, 15/54
US CL : 435/15, 194; 536/23.2
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/15, 194; 536/23.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 860 506 A1 (SMITHKLINE BEECHAM CORPORATION) 26 August 1998, see particularly pages 9 and 10.	1-3
X	US 5,965,420 A (CREASY ET AL.) 12 October 1999, see columns 13 and 14.	1-3
X	GARRET, S. et al. The Saccharomyces cerevisiae YAK1 Gene Encodes a Protein Kinase That is Induced by Arrest Early in the Cell Cycle. Mol. Cell. Biol. August 1991. Vol. 11, No. 8, pages 4045-4052, see entire document.	1-3

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
29 JANUARY 2001

Date of mailing of the international search report
19 MAR 2001

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